Rapid Genetic Decline in a Translocated Population of the Endangered Plant *Grevillea scapigera*

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Abstract: Grevillea scapigera *is one of the world's rarest plant species, currently known from only five plants in the wild. In 1995, 10 plants were selected from the 47 plants known at the time to act as genetically representative founders for translocation into secure sites. Ramets were micropropagated and introduced into one of these secure sites (Corrigin) in 1996, 1997, and 1998. By late 1998, 266 plants had been successfully translocated and were producing large numbers of seeds. With the development of an artificial seed-germination technique and because of an absence of seed germination in situ, seed was collected from these plants and germinated ex situ, and 161 seedlings were returned to the field site in winter 1999. We used the DNA fingerprinting technique of amplified fragment-length polymorphism (AFLP) to (1) assess the genetic fidelity of the clones through the propagation process, (2) contrast genetic variation and average genetic similarities of the F1s to their parents to assess genetic decline, and (3) assign paternity to the reintroduced seeds to assess the reproductive success of each clone. We found that 8 clones, not 10, were present in the translocated population, 54% of all plants were a single clone, and the F1s were on average 22% more inbred and 20% less heterozygous than their parents, largely because 85% of all seeds were the product of only 4 clones. Ultimately, effective population size (N_e) of the founding population was approximately two. Our results highlight the difficulty of maintaining genetic fidelity through a large translocation program. More generally, rapid genetic decline may be a feature of many translocated populations when N_e is small, which may ultimately threaten their long-term survival. Strategies to reverse this genetic decline include equalizing founder numbers, adding new genotypes when discovered, optimizing genetic structure and plant density to promote multiple siring and reduce kinship, promoting natural seed germination in situ rather than artificially germinating seeds ex situ, and creating a metapopulation of numerous translocated populations to restore historical distribution patterns and processes.*

Declinación Genética Rápida en una Población Translocada de una Planta en Peligro *Grevillea scapigera*

Resumen: Grevillea scapigera *es una de las especies de plantas más raras del mundo, conocida solo por cinco plantas silvestres. En 1995, 10 plantas fueron seleccionadas de las 47 que se sabía entonces eran fundadores genéticamente representativas para translocación a sitios seguros. Se micropropagaron rámulas y fueron introducidas en uno de esos sitios seguros (Corrigin) en 1996, 1997 y 1998. A fines de 1998, se habían translocado con éxito 266 plantas y estaban produciendo abundantes semillas. Con el desarrollo de una técnica de germinación artificial de semillas debido a la ausencia de germinación in situ, se recolectaron semillas de estas plantas y semillas germinadas ex situ, y 116 plántulas fueron reintroducidas al sitio en el invierno de 1999. Utilizamos una técnica de marcaje de ADN de longitud del fragmento polimorfismo amplificado (LFPA) para (1) evaluar la fidelidad genética de los clones durante el proceso de propagación, (2) contrastar la variación genética y las similitudes genéticas promedio de las F1 con sus progenitores para evaluar la*

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Paper submitted March 5, 2001; revised manuscript accepted August 9, 2001.

declinación genética y (3) asignar paternidad a las semillas reintroducidas para evaluar el éxito reproductivo de cada clon. Encontramos que 8 clones, no 10, estaban presentes en la población translocada, 54% de todas las plantas eran de un solo clon, y las F1 tenían en promedio 22% más de consaguinidad y 20% menos de heterocigosidad que sus progenitores, principalmente debido a que el 85% de las semillas eran producto de tan solo 4 clones. Finalmente, el tamaño efectivo de la población (N_e) fundadora era de aproximadamente dos. Nuestros resultados resaltan la dificultad de mantener fidelidad genética por medio de un programa de translocación. En general, la rápida declinación genética puede ser una característica de muchas poblaciones translocadas cuando N_e es pequeño, lo que al final puede amenazar su supervivencia a largo plazo. Las estrategias para revertir esta declinación genética incluyen igualar el número de fundadores, agregar nuevos genotipos cuando sean descubiertos, optimizar la estructura genética y la densidad de plantas para promover reproducción múltiple y reducir la consaguinidad, promover la germinación natural in situ en lugar de germinar semillas artificialmente ex situ y crear una metapoblación de numerosas poblaciones translocadas para restaurar los procesos y patrones históricos de distribución.

Introduction

Translocation is a difficult and complex process involving the deliberate transfer of organisms from one place to another, including existing or new sites or those where the taxon is now extinct (World Conservation Union 1987; Akeroyd & Wyse-Jackson 1995; Falk et al. 1996; Australian Network for Plant Conservation 1997; Milton et al. 1999; Moritz 1999; Fischer & Lindenmayer 2000). The principal objective of translocation is to establish secure, self-sustaining populations of taxa otherwise threatened with extinction. For many threatened species, translocation from vulnerable to secure sites is the only option available for recovery and long-term conservation. Initial goals include successful establishment and the control of immediate ecological threats from, for example, predation, desiccation, or weeds (Bowles & Whelan 1994; Akeroyd & Wyse-Jackson 1995; Australian Network for Plant Conservation 1997; Milton et al. 1999). The risk of population extinction from these immediate threats can be largely ameliorated through the rapid increase in the numbers of individuals in a translocated population and the removal or control of predators or herbivores. This also serves to offset more intermediate threats such as demographic and environmental stochasticity. Once establishment is achieved, successful reproduction is an essential short-term goal and, for plants, includes flowering, seed-set, and natural recruitment (Morgan 2000).

In the longer term, however, the objective for self-sustaining translocated populations is the maintenance of initial levels of genetic variation, which presumably reflects that found in wild populations. For outbreeders, large effective population sizes are typically required to avoid genetic decline caused by inbreeding and/or genetic drift, and to maximize the evolutionary flexibility to respond adaptively to environmental changes (Franklin 1980; Soulé 1980; Barrett & Kohn 1991; Ellstrand & Elam 1993; Guerrant & Pavlik 1998). When inbreeding depression is associated with inbreeding, a reduction in fitness can significantly increase the extinction risk of a population (Frankham 1995, 1998; Newman & Pilson 1997; Saccheri et al. 1998). In a worst-case scenario, even a population with a large number of individuals may be effectively sterile and doomed to extinction if self-incompatibility loci are largely invariant (De Mauro 1993). Alternatively, the mixing of genetically diverse populations can lead to outbreeding depression—a reduction in the fitness of hybrids through the breakdown of coadapted gene complexes, genetic incompatibilities, or the disruption of meiosis through chromosomal differences (Templeton 1986; Fenster & Dudash 1994). Ultimately, the long-term success of translocations may be compromised by these genetic problems, which can be avoided by establishing and maintaining levels of genetic diversity that are typical for wild populations of the taxon of interest.

The Corrigin grevillea (*Grevillea scapigera*, Proteaceae) is a rare and threatened prostrate woody perennial with white, sweetly scented flowers (Olde & Marriott 1995; Rossetto et al. 1996). It is a short-lived (<10 years), postdisturbance (e.g., fire), outbreeding opportunist. As such, aboveground populations may be ephemeral yet survive dormant below ground in a large and long-lived seed bank. Seed dispersal is largely passive (gravity), and seed predation is a major source of mortality. Due primarily to extensive habitat loss—>95% of its natural habitat has been cleared for farming—*G. scapigera* is now one of the world's rarest plant species. First collected in 1954 (George 1974), the species was thought to be extinct in 1986, when the only known naturally occurring plant died. Since then, other plants have been discovered. In 1994, 27 plants from eight populations were known to occur in the wild (Rossetto et al. 1995). All known wild populations occurred in highly vulnerable, degraded, and isolated remnants of natural vegetation on road verges in a 50-km radius in the wheatbelt region of southern Western Australia. As of November 2000, only 5 wild plants were known to occur in natural population remnants. In 1994 the 27 extant plants, plus an additional 20 sampled prior to death,

were measured for genetic diversity by Random Amplified Polymorphic DNA (RAPD) (Rossetto et al. 1995). Ten plants that encompassed 87% of all RAPD-generated markers and displayed typical within-population levels of genetic variability (Rossetto et al. 1995) were selected for micropropagation (Bunn & Dixon 1992). These propagules were used to establish two translocated populations in secure sites (Corrigin Airstrip Reserve and Hartleys Reserve). Earlier translocation attempts in two other populations failed. Since then, the primary focus has been on the Corrigin site; between 1996 and 1998, hundreds of ramets (tissue-cultured propagules of these 10 clones) were introduced, and 266 ramets were surviving in January 1999.

The number of ramets of each clone introduced into the Corrigin site reflected different propagation success rates. Prior to 1998, all propagation was by tissue culture of vegetative material because seed germination was impossible (Bunn & Dixon 1992). But with the combination of new techniques to germinate seed and the production of large numbers of naturally pollinated seed at Corrigin, seeds were harvested in 1998, germinated ex situ at Kings Park, and returned to the Corrigin site in the winter of 1999.

We (1) assessed the genetic fidelity of the clones in the Corrigin site (i.e., were they correctly labeled?), which are the products of over 5 years of intensive propagation involving many researchers, community groups, and volunteers; (2) contrasted the genetic diversity of the naturally pollinated but artificially germinated F1s to their parents to assess genetic change; and (3) assigned paternity to the F1s to assess the genetic contribution of parental clones.

Ultimately, we asked whether initial levels of genetic diversity were maintained. Although the short-term objectives appear to have been met and the Corrigin population has increased in size and is successfully reproducing, we report here on a significant decline in genetic variation in just a single generation, which, if not reversed, may threaten the long-term viability of this population. We then discuss strategies to reverse this genetic decline that are generally relevant to the genetic management of translocations. Although other researchers have assessed genetic variation for founder-population composition either before or after translocation (Mistretta 1994; Rossetto et al. 1995; Robichaux et al. 1997; Guerrant & Pavlik 1998; Friar et al. 2000; Smulders et al. 2000; Young & Murray 2000), our study measures in detail realized mating patterns and the genetic makeup of both the founding plants and their offspring to assess genetic change in a translocated population.

Methods

In December 1998, we collected naturally pollinated seed from plants (ramets) representing 6 (c2, c27, c33a, c33c, c37, c96) of the 10 (also c1, c16, c23, c32) clones present in the Corrigin population (Table 1). Seeds were not collected from the latter 4 clones because these ramets were only planted in mid-1998, and, although many had flowered and potentially contributed to the pollen pool, most had produced few or no seeds.

Naturally pollinated seeds were also collected from 36 plants (ramets) representing 6 clones housed at Kings Park (c2, c16, c23, c27, c33a, c33c). A further 2 clones (c3 and c8) also occurred at Kings Park but were not sampled for seed, bringing to 12 the assumed total number of clones present at Corrigin and Kings Park. A sample of 161 seeds, such that clones were represented approximately in proportion to their maternal abundance, were germinated ex situ in a glasshouse at Kings Park and returned to the Corrigin population in the winter of 1999.

Genotyping

We extracted the DNA from fresh or frozen (−80° C) young leaves with DNAzol according to the manufacturer's (Life Technologies) instructions. When possible, at least three plants (ramets) of each clone from the Corrigin population and a random sample of 103 of the 161 seedlings returned to the Corrigin population were genotyped. Multilocus DNA fingerprints were generated for each sample by the amplified fragment-length polymorphism (AFLP) technique with fluorescently labeled primers and an ABI Prism 377 automated sequencer. The AFLP procedures were as set out previously (Krauss 1999, 2000*a*, 2000*b*), except that the primer pairs we used were m-cac/e-act, m-cac/e-agg, and m-cac/e-acc. With GeneScan and Genotyper software, we scored the AFLP profiles for the presence or absence of fragments between 70 and 500 bp.

Table 1. Numbers of ramets of each clone of *Grevillea scapigera* surviving in the Corrigin translocated population as of January 1999.

	Year planted			
*Clone**	*1996*	*1997*	*1998*	*Total (%)*
1,2,32		42	101	143 (53.8)
16			6	6 (2.2)
23			21	21 (7.9)
27		16		16 (6.0)
33c		8	41	49 (18.4)
33a	1	25		26 (9.8)
96	3			3 (1.1)
37	2			2 (0.8)
total	6	91	169	266 (100)

**Clones 1, 2, and 32 were initially thought to be distinct but were found in this study to be identical.*

Data Analysis

Genetic diversity was assessed for the parental and F1 generations by the total number of markers scored, the number and proportion that were polymorphic, and the expected heterozygosity ($H_e = 2pq$) averaged over all loci, where q was measured by the Bayesian estimate (Zhivotovsky 1999; Krauss 2000*b*). We used the coefficient of similarity to construct a similarity matrix for all pairs of samples, which yields the proportion of fragments shared between two individuals (a and b) and is calculated as

$$S_{ab} = 2N_{ab}/(N_a + N_b), \quad (1)$$

where N_a and N_b represent the total band count for individuals a and b and N_{ab} represents the total number of bands shared. This value was converted to a measure of dissimilarity (D), where $D = 1 - S$. Average dissimilarity across all pairs of individuals was calculated for parents; F1s, known half sibs; and known full sibs (following paternity assignment).

Paternity was assigned unambiguously to each seedling by paternity-exclusion analysis, according to procedures previously outlined (Krauss 1999, 2000*a*). An absence of at least two fragments was used to exclude a non-sire in all cases.

The effective population size (N_e) of the translocated population was calculated as

$$N_e = H_o/2(H_o - H_1), \quad (2)$$

where H_1 and H_o are Bayesian estimates of mean heterozygosity in the progeny and parental generations, respectively, and effective population size is the number of individuals in an ideal population that would have the same genetic response to random processes as a real population of size N (Wright 1931). Fertility variation is also a major cause of a discrepancy between N and N_e, such that

$$N_e = (Nv_k - 1)/[v_k - 1 + \mathrm{var}(k)/V_k], \quad (3)$$

where V_k is the average number of progeny per individual in a population of N diploids with variance var(k) (Hedrick 1983; Frankel et al. 1995). Maternal and paternal contributions to the progeny were used to calculate N_e from fertility variation.

Results

Parental Clones

Three AFLP primer pairs generated 143 bands of between 70 and 500 bp for the parental clones, of which 108 (75.5%) were polymorphic (Table 2). Individual clones generated DNA profiles with between 70 and 84 bands. Identical DNA fingerprints were generated for the clones labeled 1, 2, and 32, indicating that these clones were mislabeled and are in fact genetically identical. Clone 27 ramets planted in 1998 were genetically distinct from clone 27 ramets planted in 1997, but were genetically identical to clone 33c. Consequently, propagation material of clone 33c was incorrectly labeled "clone 27" at some stage between 1997 and 1998. All other clones were genetically distinct, differing by between 24 (clones 3 and 16) and 52 (clones 1, 2, 32, and 37) band polymorphisms. Ultimately, 10 clones, not 12, constituted the *G. scapigera* genetic pool for translocation. Although all 4 clones introduced into the Corrigin site were genetically distinct in 1997, only 4 clones (not the assumed 7) were introduced in 1998, because clones 1, 2, and 32 were identical and clone 27 (in 1998) was in fact clone 33c (Table 1). Consequently, until 1999, 54% of all plants (ramets) at the Corrigin translocated site were genetically identical (c1, 2, 32) (Table 1).

The Bayesian estimate of population heterozygosity, averaged over all loci, was 0.317 (SE = 0.009). Genetic dissimilarity (D) among all pairs of clones varied between 0.16 (clones 3 and 16) and 0.34 (clones 1, 2, 32, and 37), with an average of 0.266 (Table 2).

F1 Generation

In contrast to the parental clones, the 103 F1s generated 5.6% fewer bands, although an equal proportion were polymorphic (Table 2). The Bayesian estimate of heterozygosity for the F1s was 20.2% less than that for the parentals (i.e., the inbreeding coefficient $F = 1 - H_t/H_o = 0.202$), which was statistically significant ($t = 28$; $p < 0.0001$) (Table 2). Mean genetic dissimilarity (D) dropped 22.3% from founding parents ($D = 0.266$; SEM = 0.005; $n = 45$) to F1s ($D = 0.208$; SEM = 0.001; $n = 0.001$),

Table 2. Genetic statistics generated by three amplified fragment-length polymorphism primer pairs for parental clones and a sample of their naturally pollinated offspring, collected in 1998, in the Corrigin translocated population of *Grevillea scapigera*.*

	n	*No. of markers*	*No. of polymorphisms*	*Polymorphisms (%)*	H_e *(SEM)*	D *(SEM)*
Clones	10	143	108	75.5	0.317 (0.009)	0.266 (0.005)
Progeny	103	135	106	78.5	0.253 (0.014)	0.208 (0.001)

**Abbreviations:* H_e, *expected heterozygosity estimated by a Bayesian method (see text);* D, *average genetic dissimilarity between all pairs of individuals; and SEM, standard error of the mean.*

which was statistically significant ($t = 26$; $p < 0.0001$) (Table 2; Fig. 1). Average D for known half sibs ($D = 0.197$; SEM = 0.002; $n = 1539$) and full sibs ($D = 0.160$; SEM = 0.002; $n = 411$) was 74% and 60%, respectively, of D for founding parents (Fig. 1).

Parentage

Despite a planting arrangement (pairs of clones alternating in rows) that suggests high rates of self-pollination, only one seed produced no nonmaternal bands and was presumably selfed. All other seeds (102 of 103) were outcrossed (with between 5 and 15 nonmaternal bands), giving a population outcrossing rate of 99%. Parentage was dominated by four clones (Table 3; Fig. 2). Sixty percent of seedlings were mothered by two clones (clones 1, 2, 32, and clone 27), whereas 82% of all seedlings were sired by four clones (clones 1, 2, and 32 and clones 27, 33a, and 33c) (Table 3; Fig. 2). In total, 85% of the genetic contribution to the F1s was from only four parental clones. Consequently, of the 5253 possible F1 pairs, 1539 (29.3%) were half sibs and 411 (7.8%) were full sibs, as determined directly by paternity assignments (Table 3). The total number of sibs does not take into account the F1s with unknown sires and thus may be a slight underestimate. Ten seeds were sired by an unknown clone, because all known potential sires were eliminated by paternity-exclusion analysis of nonmaternal bands for these seeds. This result suggests that at least one further, as yet unidentified, clone exists in the Corrigin and Kings Park ex situ populations.

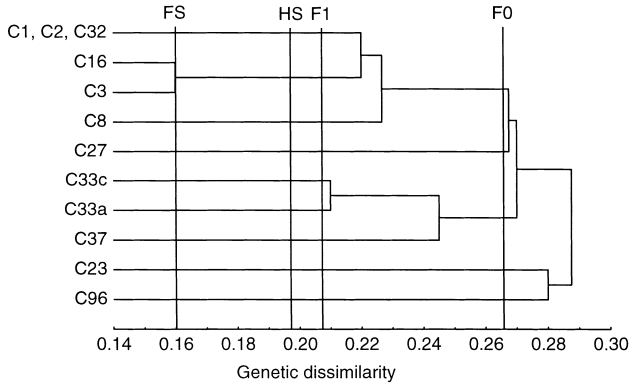

*Figure 1. An unweighted pair-group method based on arithmetic averages (UPGMA) dendrogram showing genetic dissimilarity (*D = 1 − S*, where* S *is the band-sharing coefficient) between all clones of* Grevillea scapigera *targeted for translocation. Also indicated are average genetic dissimilarities for each pair of founding clones (F0), F1s (F1), as well as half sibs (HS) and full sibs (FS) as determined by paternity assignment using amplified fragment-length polymorphism.*

Table 3. Parent assignment for each of 103 seedlings of *Grevillea scapigera* harvested from and returned to the Corrigin translocated population.

	Paternal clone									
Maternal clone	*1,2,32*	*16*	*27*	*33a*	*33c*	*23*	*37*	*96*	*?**	*Total*
1,2,32	1	3	6	6	7	2			6	31
16	1			2	2					5
27	9			8	11			1	2	31
33a	3		4		6					13
33c	2	1	9	2		1			2	17
37	1			1						2
96				2			1			3
23			1							1
?*										0
Total	17	4	20	21	26	3	1	1	10	103

**Unidentified clone(s) for which paternity analysis excluded all known potential sires.*

Effective Population Size

Substituting the heterozygosity values determined for *G. scapigera* at establishment and after one generation ($H_e = 0.317$ and 0.253, respectively) into equation 2 gives a calculated N_e of 2.5, whereas substituting fertility variation estimates for *G. scapigera* (Table 3) into equation 3 gives an N_e of 1.5.

Discussion

Rapid Genetic Decline in *Grevillea scapigera*

The short-term translocation objectives have been successfully achieved to date for the translocated population of *G. scapigera* at Corrigin. These include establishment and reproduction, so that many thousands of seeds have been generated which, for those surviving predation, have been incorporated into the long-lived seed bank. However, we detected a substantial decline in genetic variation between the founding plants and their offspring due to biparental inbreeding, which if not reversed may ultimately affect the long-term viability of the translocated population. Despite near complete outcrossing, heterozygosity decreased 20%, the number of markers amplified (an indirect measure of allelic richness) fell 6%, and the F1 generation was, on average, 22% more inbred than its parents. This striking genetic decline was largely a consequence of the variance in reproductive success, in which only four of nine clones present generated 85% of the F1s, and, as a consequence, N_e was approximately 2.

We found that 29.3% of all possible pairs of progeny were half sibs (17.3% maternal half sibs) and 7.8% were full sibs. In a related study, Krauss (2000a) found that in a small, isolated, natural population of the similarly outcrossing *Persoonia mollis* (Proteaceae), only 1.9% of all

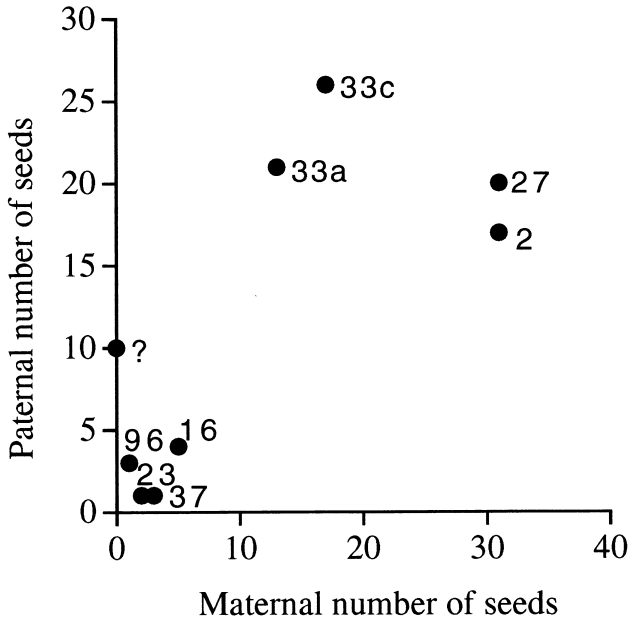

Figure 2. Maternal and paternal contribution (as determined by paternity assignment using amplified fragment length polymorphism) of each clone of Grevillea scapigera *to naturally pollinated seedlings propagated ex situ and returned to the source population at Corrigin in 1999 (? indicates unidentified clone(s), for which paternity analysis excluded all known potential sires).*

possible pairs of progeny were maternal half sibs and 0.9% were full sibs. Furthermore, there was an overall absence of inbreeding, due largely to an overall absence of population genetic structure (Krauss 2000*a*). Because *P. mollis* and *Grevillea scapigera* share similar life histories—both are insect-pollinated and fire-sensitive, such that adult plants are killed by fire and recruitment is dependent on a seed bank that responds to disturbance—it may be that natural populations of *G. scapigera* would have historically displayed patterns of realized mating similar to those of *Persoonia mollis.* It seems likely that the extent of inbreeding in the translocated *Grevillea scapigera* population is significantly higher than would be likely to occur in naturally germinated seeds in natural populations of *G. scapigera.* Similarly, the artificial overlap of generations in the translocated population of *G. scapigera*, which increases the overall population level of average genetic similarity, would not occur in wild populations because adults are killed by fire and fire is required for seed germination.

Reproductive failure and genetic decline in the translocated population is likely to accelerate in subsequent generations because 54% (143 of 266) of all vegetatively propagated ramets were of a single clone (clones 1, 2, and 32) and 90% of the population is made up of only four clones. Although the initial objective was for a more-or-less equal representation of all founding clones (Rossetto et al. 1995), difficulty in propagating some clones and mislabeling during the propagation program has resulted in a striking departure from equal representation. Consequently, the proportion of ovules converted to seeds is likely to decrease significantly because *G. scapigera* is facultatively self-incompatible with 99% outcrossing in this population, despite predominant self-pollination via geitonogamy. This is consistent with other small populations, in which size often significantly impairs seed production (Hendrix & Kyhl 2000; but see Morgan 2000). At least half of all randomly selected pairs of *G. scapigera* seed generated from 1999 onwards will be half sibs. Of the remainder, a significant proportion (likely less than the 10% detected in the 1998 cohort) will be full sibs. Consequently, the mean kinship in future cohorts will be significantly greater than detected here and is likely to increase in subsequent generations because of an increasing proportion of mating between first- and second-order relatives.

Consequences of Genetic Decline

The effects of inbreeding on plant population viability often are hard to predict (Barrett & Kohn 1991; Ellstrand & Elam 1993; Young et al. 1996). Theoretical studies and recent empirical results for outbreeding populations in the wild indicate that a decline in genetic variation due to increased inbreeding can increase the risk of population extinction (Frankham 1995, 1998; Newman & Pilson 1997; Saccheri et al. 1998). These observations apply particularly to previously widespread species that have recently become highly fragmented (Huenneke 1991).

For species that exist as naturally small and isolated populations, the consequences of inbreeding may not be so severe, even for outbreeders (Barrett & Kohn 1991; James 2000; Wolf et al. 2000). Many western Australian plants have evolved genetic and/or life-history mechanisms to survive as small, naturally fragmented and inbred populations in an ancient and relatively undisturbed landscape (Hopper et al. 1996; James 2000; Bond & Midgley 2001). Strategies include resprouting and persistence over recruitment (Bond & Midgley 2001) and complex chromosomal arrangements that maintain heterozygosity (James 2000).

However, *G. scapigera* is probably ill-adapted as a small and isolated population because it is short-lived and completely outcrossing and probably has high historical levels of gene flow despite physical fragmentation (Rossetto et al. 1995). Consequently, although it is still unclear whether the detected decline threatens the viability of the translocated population of *G. scapigera* at Corrigin, it is best to take a cautionary approach and implement steps to reverse this genetic decline.

Strategies to Prevent Genetic Decline

When population size is stable ($V_k = 2$) and fecundity is invariant (var(k) = 0), then from equation 3 $N_e = 2N - 1$ (Frankel et al. 1995). This shows that controlling the variance in both male and female reproductive success by equalizing founder numbers will double the effective size of a population (Frankel et al. 1995). With plants, it is also possible to arrange them in such a way as to maximize the number of clones in reproductive proximity and to avoid overall genetic structuring (Krauss 2000*a*). Such a design maximizes the probability of multiple siring and minimizes the mean kinship of the offspring.

However, even an equal contribution of all 10 genotypes and random mating would see an increase in inbreeding coefficient (F) of 5% in a single generation. A theoretical consideration for managing the genetic resources of outcrossing species is that natural selection for performance and fertility can balance inbreeding depression if ΔF is $<1\%$ per generation (Franklin 1980; Soulé 1980). This equates to an N_e of 50, a number that is currently impossible to achieve for *G. scapigera* because only 12 of the original genotypes (Rossetto et al. 1995) exist. Although an N_e of approximately 50 has been used as a theoretical minimum (Franklin 1980; Frankel et al. 1995), successful breeding programs (at least in the short term) have been achieved with much lower founding numbers (Hedrick & Miller 1992).

The only way to increase N_e is to use offspring of the founders, and this has been the current strategy, but this increases the mean population kinship, leading to biparental inbreeding in future generations. Translocations of rare species need to balance the genetic conflict between increasing N_e and minimizing kinship with the more immediate concerns of maximizing population size. We will continue to compare the Corrigin population with the most recently established translocated population 25 km away at Bullaring, which has been established only with the artificially propagated offspring of the founders of the Corrigin population. The Bullaring population has higher kinship and higher N_e, whereas the Corrigin site has lower kinship and lower N_e.

Recreating Historical Processes

Ultimately, the optimal strategy for the long-term conservation of genetic variation in species requiring translocation is to recreate historical processes as closely as possible (Moritz 1999). While it appears that small population size was a natural feature of *G. scapigera*, they displayed higher levels of genetic variation (Rossetto et al. 1995) than the artificially germinated offspring at Corrigin. This suggests that the decline seen in this translocated population does not occur under natural conditions in the wild. It also indicates that the soil seed bank is significantly more homozygous than the surface plant population, as might be expected for highly structured populations (Cabin et al. 1998) but not for weakly structured populations (Krauss 2000*a*). Artificial germination of a random selection of seed, although facilitating the rapid increase in numbers, may circumvent natural selection against more homozygous individuals that may occur in the wild, generating seedlings more inbred than would occur in wild populations. Consequently, a strategy to maintain genetic variation is to promote natural germination from the soil seed bank through disturbance, (e.g., burning or applying smoke) (Dixon et al. 1995).

Alternatively, selection against more inbred individuals may occur after germination. Postzygotic selection against more homozygous selfed offspring is a feature of many self-compatible plants, such as *Eucalyptus* (James & Kennington 1993; Kennington & James 1997; Potts & Wiltshire 1997). We will continue to monitor seedlings returned to the translocated populations by contrasting fitness variables to individual levels of inbreeding. Ultimately, we need to determine at what point individual inbreeding depression translates to reduced population fitness.

Perhaps more important, indirect measures suggest that historical levels of gene flow were high in *G. scapigera* (Rossetto et al. 1995). Although seed dispersal is negligible (passive gravitation), pollen dispersal by insects among populations may occur because mass flowering and a strong scent can attract pollinators over a long distance. Although the historical distribution of *G. scapigera* is unknown, it probably existed as a metapopulation of small, fragmented, ephemeral populations. This type of distribution can actually foster interpopulation gene flow (Young et al. 1996), leading to the erosion of population differentiation and the prevention of genetic decline in small, isolated populations. Consequently, recovery of *G. scapigera* should incorporate this historical metapopulation structure to facilitate historical evolutionary processes by establishing a network of translocated populations. We recommend that more populations of *G. scapigera* be established in secure sites and that routine exchange of genetic material between these populations (at >1 individual per generation; Mills & Allendorf 1996) be carried out if it is shown that pollen flow is not occurring naturally in this highly fragmented habitat.

Conclusions

Achieving short- and long-term goals for the translocation of rare plants presents considerable challenges in applying the principles of conservation genetics in practice. These challenges are particularly difficult for translocated populations of species that were historically part of a continuum which is now artificially highly fragmented. Ultimately, the emphasis for translocation must be on restoring or simulating historical processes. Popu-

lation genetics theory and some empirical data suggest that for diploid outbreeders such as *G. scapigera*, small closed populations, such as those often created by translocations, have an increased risk of extinction as a result of genetic decline through inbreeding and genetic drift. It will require careful monitoring, however, to determine whether these theoretical consequences are as severe for species such as *G. scapigera* that historically displayed a metapopulation distribution of small ephemeral populations, a long-lived, dormant seed bank, and opportunistic germination response. The flora of southwestern Western Australia includes many species with adaptations allowing them to survive ancient fragmentation and small population size (Hopper et al. 1996; Coates 2000; James 2000). More empirical data are required to understand the extinction threat posed by small population size in this unique and ancient landscape, especially for those species targeted for translocation.

Acknowledgments

This research and species recovery was funded by Environment Australia via a Natural Heritage Trust grant and supported by the World Wide Fund for Nature. We thank the many people who have assisted with the *G. scapigera* translocation project, including members of the Corrigin Grevillea recovery team, Corrigin Shire Council, Bullaring Ratepayers Association, local volunteers from the Corrigin district, and regional volunteers of the Department of Conservation and Land Management (CALM). We thank S. Hopper, M. Rossetto, K. Holsinger, and two anonymous reviewers for comments on an earlier draft of this paper and A. Bishop and W. Hudson-Taylor for lab and field assistance.

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